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Cucumis metuliferus is resistant to root-knot nematode *Mi1.2* gene (a)virulent populations and a promising melon rootstock.

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ABSTRACT

Pot experiments were carried out to characterize the response of two *Cucumis metuliferus* accessions against (a)virulent *Meloidogyne arenaria, M. incognita* or *M. javanica* populations, to *Mi1.2* gene and to determine the compatibility and the effect on physicochemical properties of cantaloupe melon. In addition, histopathological studies were conducted. Plants were inoculated in 200 cm^3-pots with 1 J2 cm^{-3} of soil containing

sterilized sand a week after transplanting and maintained in a growth chamber at 25 ºC for 40 days. The susceptible cucumber cv. Dasher II or melon cv. Paloma were included for comparison. The number of egg masses and number of eggs per plant were assessed, and the reproduction index (RI) was calculated as the percentage of eggs produced on the *C. metuliferus* accessions respect those produced on the susceptible cultivars. The compatibility and fruit quality was assessed grafting three scions (two of Charentais type) and one of type Piel de Sapo under commercial greenhouse conditions. The resistance level of both *C. metuliferus* accessions ranged from highly (RI < 1%) to resistant (1% ≤ RI ≤ 10%) irrespective of *Meloidogyne* populations. Melon plants grafted onto *C. metuliferus* accession BGV11135 grew as selfgrafted plants and did not modify negatively fruit quality traits. Giant cells induced by RKN on *C. metuliferus* were mostly poor developed compared to those on cucumber. Furthermore, necrotic areas surrounding the nematode were observed. *C. metuliferus* accession BGV11135 could be a promising melon rootstock to manage *Meloidogyne spp.* irrespective of its (a)virulent *Mi1.2* condition without melon fruit quality reduction.

Key words: *Cucumis melo*, grafting, histopathology, horned cucumber, *Meloidogyne*, plant resistance.

INTRODUCTION

Root-knot nematodes (RKN), *Meloidogyne spp.*, are the most limiting plant parasitic nematodes for vegetable production worldwide (Sikora & Fernández, 2005). Nonetheless, the capability to any RKN species to use a given plant species, to reproduce on it, and to affect its productivity differs according to its host status. Regarding cucurbit crops, one of the most widely cultivated groups around the word, zucchini-squash and watermelon

50 are susceptible and poor-host, respectively, but both are tolerant (López-Gómez *et al.*,
51 2015 & 2016), whilst melon and cucumber are susceptible and are severely damaged by
52 RKN (Di Vito *et al.,* 1983; Giné *et al.,* 2014 & 2017). In Spain, crop rotation
53 sequences including solanaceous and cucurbit crops are very common (Ornat *et al.*, 1997;
54 Talavera *et al*., 2012; Giné *et al*., 2016), but there are not available commercial resistant
55 cucurbit cultivars or rootstocks. A way to suppress RKN populations and to reduce yield
56 losses of the most susceptible-intolerant cucurbit crops by non-chemical methods,
57 according to the European directive 2009/128/CE, is grafting onto resistant-tolerant
58 rootstocks. Plant resistance is an effective and profitable control method (Sorribas *et al.*,
59 2005) reducing the RKN reproduction rate and the equilibrium density (Talavera *et al.*,
60 2009; Giné & Sorribas, 2017), and thus, the subsequent yield losses for the next crop
61 (Ornat *et al.,* 1997) which are directly related to nematode population densities in the soil
62 at planting (Seinhorst, 1965). Grafting is an effective tool for controlling other soil borne
63 pathogens (Lee *et al*., 2010). In this sense, cucurbit crops are usually grafted onto
64 *Cucurbita* hybrids, which are resistant to fusarium wilt but susceptible to *Meloidogyne*
65 *spp.* (Thies *et al*., 2010; López-Gómez *et al*., 2016; Giné *et al*., 2017). However,
66 resistance to RKN has been found in wild *Cucumis* spp., including accessions of *Cucumis*
67 *africanus, Cucumis anguria, C. ficifolius, C. metuliferus, C. myriocarpus, C. postulatus,*
68 *C. subsericeus,* and *C. zeyheri* (Fassuliotis, 1967; Sigüenza *et al*., 2005; Kokalis-Burelle
69 & Rosskopf, 2011; Pofu *et al*., 2011; Thies *et al*., 2014; Liu *et al*., 2015). Moreover, some
70 of these *Cucumis* species are resistant to pathogenic fungi, such as *Fusarium oxysporum*
71 f. sp. *melonis* (Liu *et al.,* 2015) and *Monosporascus cannonballus* (Dias *et al*., 2001). The
72 inclusion of RKN resistant cucurbit rootstocks in the solanaceous-cucurbitaceous rotation
73 sequence could be helpful to manage virulent nematode populations to *Mi1.2* resistance
74 gene on tomato, which have been increased in the last years (Tzortzakakis *et al*., 2005;

Devran & Sögüt, 2010; Verdejo-Lucas *et al*., 2012). Nonetheless, as far we know, there is no information about the host suitability of *C. metuliferus* accessions to *Mi1.2* virulent RKN populations.

Cucumis metuliferus is a compatible rootstock for melon but can affect fruit quality traits such as total soluble solids content (^{o}Brix) and flesh firmness depending on melon type and agronomic conditions (Guan *et al.,* 2014). Then, the evaluation for quality traits in different scions is convenient when testing for putative rootstocks. The objective of this study was to assess the host suitability of *C. metuliferus* against RKN (a)virulent populations, its compatibility with melon and the effects on fruit quality. In addition, histopathological studies were conducted to identify resistance mechanisms of *C. metuliferus* against *M. javanica*.

MATERIALS AND METHODS

Nematode inoculum

RKN populations belonging to *M. arenaria, M. incognita* and *M. javanica* were used in the experiments. The information on RKN species, code, origin and the (a) virulent status against tomato cultivars carrying the *Mi1.2* gene is presented in Table 1. The RKN populations were maintained on the susceptible tomato cv. Durinta (Seminis Seeds). Second stage juveniles (J2) were used as inoculum. Eggs were extracted from tomato roots by blender maceration in a 5% of commercial bleach (40 g L^{-1} NaOCl) solution for 5 min (Hussey & Barker, 1973). The egg suspension was then passed through a 74 µm aperture sieve to remove root debris, and eggs were collected on a 25 µm sieve and placed on Baermann trays (Whitehead & Hemming, 1965) at 25 °C. Nematodes were collected daily using a 25 µm sieve during 7 days and stored at 9 ºC until inoculation. *Meloidogyne*

99 species were identified according to the morphology of the perineal pattern of the females,
100 and by SCAR-PCR markers (Zijlstra *et al*., 2000).

101 *Plant material*

102 In the experiments conducted to assess the response of *C. metuliferus* against (a)virulent
103 RKN populations, accessions BGV11135 and BGV10762 of *C. metuliferus,* from the
104 COMAV-UPV collection (Valencia, Spain), and the susceptibles cucumber cv. Dasher II
105 (Seminis Seeds) or melon cv. Paloma (Fitó) were used. Seeds of *C. metuliferus* were
106 surface disinfested using a 20% bleach commercial solution (40g L^{-1} NaOCl) during 2
107 min and washed two times in sterilized distilled water to remove bleach. Afterwards,
108 seeds were placed in Petri dishes with a cotton matrix, irrigated and incubated two days
109 at 37 ºC. After germination, seeds were transferred to a tray containing sterile vermiculite,
110 covered with 1.5 cm with the same substrat and placed in a growth chamber at 25±2 ºC
111 and 16:8 h (light:darkness) photoperiod for a week. After that, seedlings were individually
112 transplanted to 200 cm^3 pots containing sterile river sand. Plants were fertilized with a
113 slow release fertilizer (15% N + 9% P2O5 + 12% K2O + 2% MgO2 + microelements;
114 Osmocote Plus).

115 To assess *C. metuliferus* compatibility with melon, Charentais melon (*Cucumis*
116 *melo* L. var. *cantalupensis* Naudin) cv. Vedrantais (COMAV) and cv. Paloma (Fitó), and
117 Piel de sapo melon (*Cucumis melo* L. var. *inodorus* Naudin) cv. Finura (Rijk Zwaan) were
118 used.

119 *Response of* C. metuliferus *accessions to avirulent RKN populations*

120 Two experiments were carried out to evaluate the response of *C. metuliferus* against
121 avirulent RKN populations. In the first experiment, accessions BGV11135 and
122 BGV10762 of *C. metuliferus,* and cucumber cv. Dasher II were inoculated with 1 J2 cm^{-3}
123 of soil of the *M. incognita* population Agropolis or the *M. javanica* population MJ05.

Plants were maintained in a growth chamber at 25±2 ºC and 16:8 h (light:darkness) photoperiod for 40 days. Plants were watered as needed during the experiment. Each plant-RKN population combination was replicated 10 times. Soil temperatures were recorded daily at 30-min interval with a PT100 probe (Campbell Scientific Ltd) placed into the pots at 4 cm depth. At the end of the experiment, roots were carefully washed, weighted and immersed in a 0.01% solution of erioglaucina to stain egg masses in blue (Omwega *et al.,* 1988) previous to count them. RKN eggs were extracted from roots by maceration in a 10% bleach commercial solution (40g L^{-1} NaOCl) (Hussey & Barker, 1973). The number of eggs was counted and the reproduction index (RI) was calculated as the percentage of the number of eggs per plant in the experimental accession with respect to that on the susceptible cucumber cv. Dasher II. After that, the response of the accessions was categorized according to the RI as, highly resistant (RI < 1%), resistant (1% ≤ RI < 10%), moderately resistant (10% ≤ RI < 25%), slightly resistant (25% ≤ RI < 50%) or susceptible (RI ≥ 50%) (Hadisoeganda & Sasser, 1982).

In the second experiment, the response of the *C. metuliferus* accession BGV11135 and the susceptible standard cucumber cv. Dasher II was assessed against one population of *M. arenaria* (MA68), two populations of *M. incognita* (Agropolis and Garriga) and three populations of *M. javanica* (Bay, MJ05 and Tugues). Each plant-RKN population combination was repeated 7 and 8 times in the first and second experiment, respectively. The experimental procedures and assessments were those described previously. The experiment was carried out twice

Response of C. metuliferus *BGV11135 to virulent RKN populations*

The response of the *C. metuliferus* accession BGV11135 and the susceptible melon cv. Paloma was assessed against three *Mi1.2* virulent RKN populations belonging to *M. arenaria* (MAAl06), *M. incognita* (MIAl15) and *M. javanica* (MJ27) in 200 cm^{-3}-pot

149 experiments. The avirulent *M. javanica* population MJ05 was included as standard for
150 comparison. The experiment was repeated once. Each plant-RKN population
151 combination was repeated 8 times each experiment. The experimental procedures and
152 assessments were those described previously.

153 *Histopathology*

154 Seeds of *C. metuliferus* BGV11135 and cucumber cv. Dasher II were surface sterilized
155 and incubated as previous to be transferred to transparent envelopes with sterilized paper
156 to maintain humidity for suitable root growth and incubated at 25 ± 2 ºC and 16:8 h
157 (light:darkness) photoperiod. Plantlets were inoculated at two true leaf expanded stage
158 with 2500 J2 of *M. javanica* MJ05. After 12 days, roots were carefully washed and cut in
159 pieces of 10 mm. Then, roots containing galls were selected and fixed in 2.5% (v/v)
160 glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) overnight at 4°C and washed
161 three times with same buffer. Afterwards, root pieces were post-fixed in 1% (w/v)
162 osmium tetroxide in 0.1 M sodium phosphate buffer (pH 7.2) for 1 h and washed three
163 times with the same buffer and dehydrated in an acetonitrile series (30–100%) before
164 embedding in epoxy resin (Embed 812, Aname®) and polymerizing at 60ºC for 48h.
165 Semithin (2µm) sections of samples were obtained in a Reichert-Jung Ultracut E Ultra
166 Microtome Leica EM UC6 (Leica Microsysteme GmbH Wien, Austria) and left to dry on
167 a slide previous to be stained with Richardson's blue. The sections were mounted in DPX
168 mountant for histology and observed under a Leica DM4000 B microscope (Leica
169 Microsystems, Mannheim, Germany). Afterwards, sections were photographed using a
170 Leica DFC300 FX 1.4-megapixel digital colour camera equipped with the Leica software
171 application suite LAS V3.8 (Leica Microsystems).

172 *Compatibility and fruit quality assessment*

The performance of *C. metuliferus* BGV11135 as rootstock was evaluated using the cv. Vedrantais (COMAV) and Paloma (Fitó) of Charentais melon (*Cucumis melo* L. var. *cantalupensis* Naudin) and cv. Finura (Rijk Zwaan) of Piel de sapo melon (*Cucumis melo* L. var. *inodorus* Naudin) as scions. Plants were selfgrafted and grafted onto *C. metuliferus* BGV11135 using the cleft procedure (Lee *et al*., 2010). Plants were grown under hydroponic conditions in a commercial greenhouse at Fundación Cajamar (Paiporta, València) during the spring-summer of 2017. In order to evaluate the impact of grafting on fruit quality, each fruit (8 per treatment) was characterized for the following traits: weight, length and width, rind and flesh thickness, rind and flesh firmness (measured with a digital Penetrometer (8 mm) FHT-803®, Melrose, MA), pH (measured with pH-indicator paper pH1-14 Merck, Darmstadt, Germany), total soluble solids (quantified through a digital Rephractometer Atago®, Tokyo, Japan), and flesh color (measured with a colorimeter, Minolta CR-400, New Jersey, USA using the color parameters Hunter L, a and b).

Statistical analysis

Analysis of variance was performed using SAS system V9 (SAS Institute, Inc., Cary, NC, USA). Data on number of eggs masses and eggs per plant were transformed to log10 (x+1) when needed to normalize them. The repetitions of the same experiment were compared by the proc glm procedure and consider as the same experiment if no differences were found. Means were separated by the least significant differences (LSD) test when statistical analysis was significant ($P < 0.05$). Paired comparisons between each grafted and selgrafted cultivars for fruit quality traits were done by Student t-test.

RESULTS

Response of C. metuliferus *accessions against avirulent populations of* Meloidogyne spp

Both *C. metuliferus* accessions (BGV11135 and BGV10762) responded as highly resistant (RI < 1%) or resistant (1% ≤ RI ≤ 10%) to RKN depending on the nematode population. The number of egg masses and eggs per plant material on both *C. metuliferus* accessions were significantly less ($P < 0.05$) than on the cucumber cv. Dasher II irrespective of the *Meloidogyne* specie (Table 2) or the populations assessed (Table 3).

The infective and reproductive capacity of *Meloidogyne* populations differed ($P < 0.05$) on both *C. metuliferus* BGV11135 and cucumber cv. Dasher II (Table 3). The nematode populations Agropolis and Garriga of *M. incognita*, and MJ05 of *M. javanica* produced the highest number of egg masses on *C. metuliferus,* whilst *M. arenaria* population MA68 did on cucumber cv. Dasher II. Regarding RKN reproduction, the *M. incognita* population Garriga produced more eggs ($P < 0.05$) than the remaining RKN populations on *C. metuliferus* whilst populations Agropolis and Garriga did on cucumber cv. Dasher II. The accession BGV11135 of *C. metuliferus* performed as resistant against the most RKN populations assessed.

Response of C. metuliferus *against virulent Mi1.2 populations of* Meloidogyne spp

C. metuliferus accession BGV11135 responded as highly resistant (RI < 1%), resistant (1% ≤ RI ≤ 10%) or moderately resistant (10% ≤ RI < 25%) to RKN, depending on the nematode population assessed irrespective of its *Mi1.2* gene (a)virulent condition (Table 4).

Compatibility and fruit quality assessment

C. metuliferus used as rootstock did not affect plant growth of Charentais and Piel de Sapo melons. Grafted plants of each cultivars showed similar vine vigour and flowering time than their respective selfgrafted plants. There was no effect of the rootstock on fruit

external and internal quality in the two Charentais melons cultivars, except from a slight increase of flesh thickness in cv. Paloma (Table 5). Each grafted Charentais cultivar maintained fruit size, rind and flesh firmness, and flesh quality (º Brix, pH and colour). Grafting the Piel de sapo melon cv. Finura onto *C. metuliferus* increased the fruit weight and length, but were softer, sweeter and the flesh with lighter colour respect selfgrafted plants (Table 5).

Histopathology

M. javanica population MJ05 induced giant cells in both *Cucumis* species (Figure 1) but those produced in *C. metuliferus* were mostly poor developed with multiple vacuoles compared to those on cucumber. Furthermore, giant cells without cytoplasm and necrotic areas surrounding the nematode were observed.

DISCUSSION

The *C. metuliferus* accessions assessed in this study performed as highly resistant (RI < 1%) or resistant (RI = 1% - 10%) to the most RKN populations. These results are in agreement with those reported previously (Fassuliotis, 1967 & 1970; Sigüenza *et al.*, 2005; Walters *et al.*, 2006; Thies *et al.*, 2014; De You-Ye *et al*., 2017). The host suitability of *C. metuliferus* was not affected by the (a)virulent condition of the nematode population. Then, it could be a useful tool to manage RKN nematodes and to prevent the selection of virulent populations in cropping sequences with resistant tomato cultivars or rootstocks. In addition, the *C. metuliferus* accessions assessed in this study are highly resistant to fusarium wilt (Gisbert *et al.*, 2014), and tolerant to *Monosporascus cannonballus* in field conditions (Perpiñà *et al.*, com pers).

Fassulotis reported the resistance response of *C. metuliferus* accession C-701 to *M. incognita* in 1967 and conducted histopathological studies in 1970, who observed

small giant cells affecting nematode development and increasing the proportion of males. However, no hypersensitive response was observed. Similar results were found by Walters *et al.,* (2006) in the accession PI482454 inoculated with *M. arenaria, M. hapla, M. incognita* or *M. javanica.* Recently, Ye *et al.,* (2017) have reported a reduction of the number of J2 of *M. incognita* in roots of *C. metuliferus* accession PI482443 at 7 than at 4 days after inoculation (dpi), indicating death or emigration from roots and a delayed development of those remaining in them. Empty or poor developed giant cells with multiple vacuoles were observed at 7 and 14 dpi, giant cells appeared to be collapsed or without cytoplasm. In addition, several genes related to plant defence mechanisms were significantly modified and, in contrast with previous reports, hypersensitive necrosis was observed. The results of this study are consistent with those previously reported, in which giant cells produced were multivacuolated and some of them surrounding the nematode area appeared collapsed without cytoplasm. Furthermore, necrotic areas were observed. These results could indicate that the *C. metuliferus* genetic background could play an important role in the interaction with *Meloidogyne sp.*

Grafting can affect fruit quality depending on the rootstock-scion interactions, climatic and agronomic conditions (Leonardi *et al.*, 2017). For instance, fruit melons of cv. Supermarket or cv. Proteo grafted onto *C. metuliferus* contained less º Brix than the ungrafted plants in one out two cropping seasons (Trionfetti-Nisini *et al.,* 2002). Guan *et al.,* (2014) reported less º Brix content and flesh firmness in galia melons but not from honeydew melons grafted onto *C. metuliferus* conducted in a conventional manner, but not under organic farming. In this study, no differences were found on growth or fruit quality from selfgrafted cantaloupe melon cv. Vedrantais and cv. Paloma with those grafted onto *C. metuliferus*. These results are in agreement with those reported by Gisbert *et al.* (2017) who did not find differences among fruit quality from ungrafted, selfgrafted

or grafted cv. Vedrantais onto *C. metuliferus*. Conversely, grafted melon Piel de sapo cv. Finura onto *C. metuliferus* affected fruit weight and length. Nonetheless, these changes do not reduce the commercial value of the fruits as the market of Piel de sapo melons accept a wide range of melon sizes and variability in shapes. The changes in parameters associated with flesh quality (higher º Brix, lower flesh firmness and lighter flesh color) might be associated to a more advanced ripening state of the grafted melons onto *C. metuliferus*. Effects on fruit quality in grafted plants due to growing cycle alterations have been reported previously (Davis *et al*., 2008; Soteriou *et al.*, 2014). Therefore, these effects could be reduced adapting the harvesting period for each rootstock-scion combination.

In conclusion, *C. metuliferus* accession BGV11135 could be a promising melon rootstock to manage *Meloidogyne spp.* irrespective of its (a)virulent *Mi1.2* condition without melon fruit quality reduction.

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